Turkey meningo-encephalitis (TME) was first described in Israel and has not been recorded elsewhere (Komarov & Kalmar, 1960). It is a neuroparalytic disease of turkeys that, under field conditions, affects birds older than 10 weeks (Ianconescu, 1975). Affected birds show a progressive paresis and paralysis. At the onset they walk as if intoxicated and later sit or lie down. They are unwilling to move but, when they are disturbed, their movements are uncoordinated. The seasonal outbreaks in Israel cause a high morbidity and mortalities up to 80%. Under natural conditions the turkey is the only species known to be affected by the virus classified as a flavivirus (Porterfield, 1961), although the virus can be propagated in vivo in mice and Japanese quail. Other birds such as chickens, ducks and pigeons are not susceptible (Komarov & Kalmar, 1960; Ianconescu, Aharonovicli & Samberg, 1974). After natural or experimental infection with TME virus, a lifelong immunity develops. Antibodies against TME can be demonstrated by virus neutralization or haemagglutination-inhibition tests.

During the autumn and early winter of 1978 a disease similar to TME was observed in turkeys on the farm “Weltevreden” in the Krugersdorp district in South Africa. A virus was isolated from diseased turkeys and a study made of the aetiology, clinical manifestation, histopathology and some aspects of the epizootiology of the disease. These aspects were compared with those of TME and the results of the investigation are presented in this paper.

Material and Methods

Turkeys

Day-old to 45-week-old turkeys were obtained from the infected farm and kept in isolation until used for different experiments. Each bird was tested for the absence of haemagglutination inhibition (HI) antibodies against the isolated virus before being used in different studies.

Prototype virus

For comparison with the local isolate the H3 strain of TME virus, which has been isolated from a turkey and passed 3 times intracerebrally in mice, was obtained from Israel(1). On receipt it was passaged once more in baby mice and stored as a freeze-dried suspension of 10% infective mouse brain in phosphate buffer containing 1% peptone, 15% lactose, 500 units of penicillin and 500 micrograms of streptomycin per ml (BLP).

Isolation and cultivation of virus

Primary isolation was performed in cultures prepared from the ovary of a diseased turkey. Subsequent isolations were performed in embryonated hen's eggs and intracerebrally in baby mice.

Five to 12-day-old embryonated hen's eggs were inoculated with 0.2 ml of a 1:10 suspension in BLP of infected turkey brain, liver, spleen, kidney and ovary, mouse brain or chorio-allantoic membranes. The yolk sac, chorio-allantoic and allantoic routes of inoculation were used. Nine-day-old embryonated hen's eggs were also inoculated intravenously. The eggs were candled daily and subcultures made when necessary.

One- to 3-day-old mice were injected intracerebrally and intraperitoneally with a 1:10 suspension of suspected or infected tissues in BLP. The mice were observed daily for 14 days. Brains of mice that died were collected and either stored at -20 °C or processed for further tests. The latter mouse brain-adapted virus was also cultured on monolayers of BHK21 cells in roller tubes and roller bottles.

Serological tests

The haemagglutination-inhibition technique (HI) of Clarke & Casals (1958) was used to determine antibody titres. A sucrose-acetone extract of infected mouse brain was used as antigen and goose red cells were used in the test which was performed at pH 6.4.

(1) Kindly supplied by Dr M. Ianconescu of the Kimron Veterinary Institute, Beit Dagan, Israel

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FIG. 1 Egg production and mortality in an infected flock
For the virus neutralization tests (SVN) tenfold virus dilutions were mixed with a constant volume of serum diluted 1:10. The serum-virus mixtures were incubated for 30 min at 37°C, inoculated onto BHK21 cell cultures in roller tubes and inspected daily for cytopathic effects (CPE).

Pathology

Several naturally infected birds as well as experimentally infected birds were examined soon after death and the results recorded.

Specimens of brain, liver, spleen, ovary, lung, myocardium and pectoral muscles were collected for histopathological and virological examination. The organs collected for histopathological examination were fixed in 10% buffered formalin, routinely processed and embedded in paraffin wax. Sections of 3-4 μm were cut and stained with haematoxylin and eosin.

RESULTS

History

The first indication of the disease in South Africa was found during April 1978, when four 32-week-old females of a female breeding line housed in an open pole-barn type laying house were found dead. Two weeks later losses were encountered in another breeding line which had been kept in the same house and one week after that the first losses occurred in a 43-week-old flock penned in an identical adjacent house. No losses were encountered amongst younger birds kept in environmentally controlled houses. In the 32-week-old flock losses were experienced for 5-6 weeks (Fig. 1), while in the 43-week-old flock losses continued for at least 9 weeks. Losses amongst females of the 32-week-old flocks were 28.7%, and 54.2% respectively, while the mortality amongst the males was only 2.5% and 1.9% respectively. In the 43-week-old flock 33.3% females and 4.1% males of the female line died while male line losses were 20.5% and 9.7% respectively.

A significant drop in egg production occurred in affected breeding flocks (Fig. 1). Fertility and hatchability of eggs laid during the period of reduced production was normal and no eggshell abnormalities were observed. Egg production of recovered hens returned to the expected level (Fig. 1).

Symptoms

Naturally affected groups of turkeys were quieter than normal. As the disease progressed, both naturally and experimentally affected turkeys showed unrectangular gait. The mortality amongst the males was only 2.5% and 1.9% respectively. In the 43-week-old flock 33.3% females and 4.1% males of the female line died while male line losses were 20.5% and 9.7% respectively.

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Intracerebrally infected turkeys showed more pronounced symptoms than birds infected by any other route. No difference was observed between turkeys infected with the local virus strain and the H₃ viral strain.

Virus isolation

An organ culture prepared from the ovary of an affected turkey developed slowly and small groups of epithelial-like and fibroblast-like cells were observed after 5 days. On the 8th day 3 foci were observed, showing CPE characterized by rounding and detachment of cells. Both epithelial- and fibroblast-like cells were involved. The CPE occurred locally during the 42 days of observation. Chicken embryos infected intravenously with culture medium collected on Day 8 did not die, but appeared stunted. Dead embryos were encountered on subsequent passages. Infant mice, injected with culture medium collected on Days 8 and 42 respectively, either died or showed paralysis within 9 days. Six-week-old turkeys injected intramuscularly or intranasally with brain suspensions of the 3rd passage in mice developed symptoms indistinguishable from those observed in turkeys during the outbreak of the disease.

Host range of virus

Turkeys. Turkeys of all ages proved their susceptibility to artificial infection by a rise in body temperature, incoordination in some birds, a drop in egg production in laying birds, and deaths. Turkeys under 6 weeks of age were more severely affected and more deaths occurred in these young birds. A biphasic temperature reaction was observed. The highest temperatures were recorded during Days 4-7 and 11-12 respectively (Fig. 2). Egg production stopped during the first peak and was not resumed 14 days later when the experiment was terminated. The same symptoms were observed in turkeys whether infected intracerebrally, intramuscularly or intranasally.

Mice. Only infant mice injected intracerebrally showed symptoms. On primary isolation of the virus the mice showed paralysis and died between the 7th and the 9th days. On subsequent passages they died between the 3rd and the 6th days.

Embryonated hen's eggs. Embryos injected intracerebrally or by the chorio-allantoic route died regularly 4-9 days after injection. Dead embryos were cherry red, with petechial haemorrhages. Only sporadic mortalities were observed after injection of embryos via the yolk sac and allantoic routes.

BHK21 cell cultures. Both the local strain and the H₃ strain showed CPE after 1 blind passage on BHK21 cell cultures. Cytopathic changes were observed on the 2nd day and more than 75% of the cells were affected by the 3rd day after inoculation. A virus titre of 10⁸ plaque forming units/ml was obtained with both viral strains. Turkeys injected with BHK21 cell suspensions developed typical symptoms.

TABLE 1 Comparison of the H₃ and the "Weltevreden" virus strain by haemagglutination-inhibition and serum virus neutralization tests

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Serology

When the first symptoms were noticed in the flock some females already had HI antibodies against TME virus. Four weeks later more than 90% of the females and 65% of the males were positive with HI antibody
FIG. 2 Temperature reaction in turkeys infected with turkey meningo-encephalitis virus. Vertical lines illustrate variation in group titres varying between 1:80 and 1:5120. The results obtained in the cross-HI and the SVN tests indicate a complete cross-reaction between the 2 strains in the HI test and both viruses were neutralized to the same extent by sera prepared against each virus (Table 1).

Gross pathology

Birds submitted or sacrificed for necropsy were in good condition. Soiled feathers were frequently present round the cloaca and a slight catarrhal enteritis was present in all the cases. Petechial haemorrhages on the peritoneal surfaces were prominent in the cases that died naturally. Natural and experimental cases showed an acute purulent peritonitis with regression of the ovaries, hepatomegaly, atrophy of the spleen and hydropericardium. In some cases the follicles were ruptured and the yolk dispersed throughout the peritoneal cavity. In most of the natural and
experimental cases, ill-defined focal greyish areas 4–8 mm in diameter were noticed in the myocardium. Some birds had focal whitish-grey areas 10–50 mm in diameter in the superficial pectoral muscles. These areas stretched into the muscle towards the clavicula.

Histopathology

Brain. Moderate meningo-encephalitis was a constant feature of all cases. The meninges were thickened (Fig. 6) and infiltrated by lymphocytes and macrophages. Extensive perivascular cuffing with lymphocytes and macrophages occurred throughout the brain (Fig. 7). Disseminated focal gliosis occurred in most cases and neuronophagia was prominent in areas of gliosis. Slight brain oedema most conspicuous in the cerebellum, was present in every case. Although the lesions occurred throughout the brain, they were more pronounced in the brain-stem.

Heart. Heart lesions were present in every case examined, although they were not confined to any specific site. Lesions varied from a focal disseminated acute myocardial necrosis in some cases to a subacute necrosis in others (Fig. 8). The areas of acute necrosis were characterized by a few necrotic muscle fibres which showed complete loss of striation and a more pronounced eosinophilic staining. Slight interstitial oedema and occasional fragmentation of the muscle fibres were also observed. The subacute cases showed extensive myocardial necrosis with a marked infiltration of macrophages and lymphocytes into the interstitium.

Skeletal muscle. Focal areas of extensive degeneration and necrosis together with early mineralization and proliferation of sarcolemmal nuclei occurred in the superficial pectoral muscles of a small percentage of the birds. The muscle fibres in these areas appeared homogenously eosinophilic with a complete loss of striations. Some of these fibres showed myolysis. A few heterophils infiltrated between the muscle fibres.

Spleen. The white pulp of the spleen was atrophic. Only a few lymphocytes were present in the germinal centres, some of which showed karyorrhexis. The reticulo-endothelial cells of the red pulp appeared prominent.

Liver. The external surface of the liver capsule was invariably thickened and covered with exudate which consisted of a few macrophages mixed with egg yolk droplets. The portal tracts were infiltrated by a few lymphocytes and macrophages. Single cell necrosis of the hepatocytes occurred in some livers.

Intestinal tract. The serosa of the gut was thickened by the infiltration of macrophages and the deposition of egg yolk droplets on this layer. The whole gastrointestinal tract was congested.

Kidney. Most cases showed a slight nephrosis. Urate crystals were occasionally found in collecting tubules.

Pulmonary. The lungs were congested and slightly oedematous in all the cases. A slight infiltration of lymphocytes and macrophages occurred around the larger bronchi.

Ovary. Ovaries were congested and atrophic in all the cases. No active follicles were seen, but there was a large number of atretic follicles present. Rupture of some of the larger follicles was a regular finding. The stroma of the ovaries was infiltrated by lymphocytes and macrophages.

Discussion

South Africa is the first country to report the isolation of a pathogenic virus for turkeys that cross-reacts with TME virus isolated in Israel in the HI and SVN test. The similarity in host range, symptoms, and pathological changes produced by the local viral strain and the H3 strain of TME virus and the serological cross-reactions indicate that they are identical. According to Komarov & Kalmar (1960) and Ianconescu et al. (1972), turkeys infected with TME virus showed a paresis followed by paralysis. The most constant symptom observed in this investigation was incoordination manifested by an unsteady gait and/or drooping of the wings. Although turkeys in extremis appeared to be paralysed, they were still able to lift their heads or flap their wings when handled. The symptoms and lesions observed in both natural and experimental cases were similar in birds of different ages.

The only macroscopic lesions mentioned by Komarov & Kalmar (1960) and Ianconescu (1975) were hypertrophy of the spleen and a catarhal enteritis in 2% of cases. In the present investigation, however, degeneration of the cardiac muscle, atrophy of the spleen, regression of the ovary and egg-peritonitis were constant findings.

All the cases examined histologically showed degeneration and necrosis of cardiac muscle, meningo-encephalitis, atrophy of the spleen and degeneration of the ovaries in laying birds. Only catarhal enteritis and meningo-encephalitis are mentioned by Ianconescu (1975). It would be interesting to know whether or not regression of the ovary and myocarditis were observed in Israel, and, if not, what are the reasons for the difference between the observations in the two countries. The pathogenesis of the degeneration and necrosis of the pectoral muscles in a small percentage of birds is unknown.

During autumn large numbers of biting insects are usually found in South Africa. The outbreak of the disease occurred during this season but only turkeys in open laying houses contracted it. Turkeys in any one house did not become diseased simultaneously and birds in certain pens ailed before others. Turkeys, which at that stage were penned in another open house 1 km distant, did not become diseased. These observations and the fact that turkeys could be infected intranasally indicate the likelihood of both contact and insect transmission. Ianconescu (1975) could not infect turkeys intranasally, but mentioned contact transmission in injured birds.

Although regression of ovaries was a constant finding and a dramatic drop in egg production occurred, it appears that the virus does not pass through the egg, as the hatchability of eggs laid during the outbreak was unaffected, and turkeys hatched from such eggs not only showed no symptoms of virus infection but were also free from antibodies against the virus.

HI antibodies developed within the first 2 weeks after infection, and the fact that within 4 weeks after the onset of symptoms in the flock more than 90% of the sera tested were positive indicated a fast spread and high morbidity.

With a mortality of up to 54% in females and 9.7% in males and a drop in egg production over a period of up to 9 weeks, the immediate loss is dramatic. When such an outbreak occurs in breeding lines, an entire production programme is seriously affected.
FIG. 3 Affected turkey with a bilateral drooping of wings
FIG. 4 Affected turkeys often sit or lie down
FIG. 5 In the terminal stage of the disease affected birds appeared to be paralysed but they are still able to lift their heads and move their legs and wings when disturbed
FIG. 6 Thickenening of meninges with mononuclear cell infiltration and oedema. HE×200
FIG. 7 Extensive perivascular cuffing with lymphocytes and macrophages occurred throughout the brain. HE×200
FIG. 8 Subacute diffuse myocardial necrosis. Note mononuclear cell infiltration. HE×200
TURKEY MENINGO-ENCEPHALITIS IN SOUTH AFRICA

Although no new cases have been encountered since August 1978 this virus remains a potential danger and warrants further investigation.

REFERENCES